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Prevalence of Antibodies to *Neospora caninum* and *Sarcocystis neurona* in Sera of Domestic Cats From Brazil

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ABSTRACT: Antibodies to *Neospora caninum* and *Sarcocystis neurona* were determined in serum samples of 502 domestic cats from Brazil using direct agglutination tests with the respective antigens. Antibodies to *S. neurona* were not found in 1:50 dilution of any serum in the *S. neurona* agglutination test, suggesting that domestic cats from São Paulo city were not exposed to *S. neurona* sporocysts from opossums. Antibodies to *N. caninum* were found in 60 (11.9%) of 502 cats with titers of 1:40 in 36 cats, 1:80 in 18 cats, 1:160 in 5 cats, and 1:800 in 1 cat using the *Neospora* agglutination test (NAT). Antibodies to *N. caninum* were confirmed by Western blotting in the sera of 10 cats with NAT titers of 1:80 to 1:800; this finding suggests that at least 10 cats had *N. caninum*–specific antibodies confirmed by 2 tests. This is the first documentation of natural exposure of cats to *N. caninum*.

Cats (*Felis domesticus*) are considered hosts to 3 related apicomplexans, *Toxoplasma gondii*, *Sarcocystis neurona*, and *Neospora caninum*; all 3 protozoans have a wide host range (Dubey and Beattie, 1988; Dubey and Lindsay, 1996; Dubey et al., 2001). There are numerous sero-epidemiological studies documenting the essential role of cats in the life cycle of *T. gondii* (Dubey and Beattie, 1988). Recently, cats were found to be an intermediate host for *S. neurona*, which is an important cause of a neurological disease of horses (Dubey et al., 2000; Dubey et al., 2001; Turay et al., 2002). Although there is no documented clinical case of *N. caninum* infection and no information on the prevalence of the parasite in cats, they can be successfully infected with *N. caninum* in the laboratory (Dubey and Lindsay, 1989a, 1989b). The objective of the present study was to determine the prevalence of antibodies to *S. neurona* and *N. caninum* in sera of naturally infected domestic cats.

Sera were collected during 1993–2000 from 502 domestic cats (*F. domesticus*) from São Paulo and Guarulhos cities, São Paulo State, in Brazil (Silva et al., 2002). A total of 470 stray cats was captured by the Center for Zoonosis Control of São Paulo and Guarulhos. Thirty-two cats were from a breeder in São Paulo; 26 of the 32 were born in houses and 6 were stray.

Cats were physically or chemically restrained using ketamine and xylazine. Blood samples were collected from each animal, and sera were stored at −20 C until serologic analysis. Antibodies to *N. caninum* were determined by the *N. caninum* agglutination test (NAT) as described by Romand et al. (1998). Sera were initially screened at the Beltsville laboratory at 1:25 dilution by NAT. Sera with positive or doubtful results were shipped to the Paris laboratory, where sera were diluted to 1:20, 1:40, 1:80, 1:160, 1:400, and 1:800, and examined by NAT (Romand et al., 1998).

Western blots were performed on cat sera that were positive at greater than 1:80 by NAT. Four to 12% gradient Bis–Tris gels containing detergent-extracted NC-1 tachyzoite proteins were electrophoresed in 50-mM MOPS-sodium dodecyl sufate running buffer. Electroblotting was carried out by transfer of proteins onto nylon blotting membranes. The membranes were saturated with 1% nonfat dry milk solution in Tris-buffered saline and incubated in 1:100 dilution of cat sera, followed by goat anti-cat antibodies conjugated to horseradish peroxidase. Bands were visualized using 4-CN substrate kit (Kirkegaard and Perry, Gaithersburg, Maryland). Pre- and postinfection sera (20 days postinoculation) from pregnant cat no. 1, inoculated subcutaneously with 2×10^6 *N. caninum* tachyzoites, were used as negative and positive controls (Dubey and Lindsay, 1989b).

Antibodies to *S. neurona* were determined by the *S. neurona* agglutination test (SAT) as described by Lindsay and Dubey (2001). The SAT was performed at 1:50 dilution at the Blacksburg laboratory (Lindsay and Dubey, 2001).

Antibodies to *S. neurona* were not found in 1:50 dilution of serum of any cat. This result was unexpected because cats fed *S. neurona* sporocysts developed high (≥1:4,000) antibody titers in SAT (Dubey et al., 2002), and SAT antibodies were found in 13% of 310 feral cats from Ohio (Stanek et al., 2003). The cats in the present study were city cats and probably had little chance to be exposed to opossum feces infected with *S. neurona* sporocysts.

Antibodies to *N. caninum* were found in 104 cats with titers of 1:20 in 44 cats, 1:40 in 36 cats, 1:80 in 18 cats, 1:160 in 5 cats, and 1:800 in 1 cat. Western blot analysis of these cat sera revealed antibody binding to *N. caninum* proteins in sera from 10 animals whose NAT titer was at least 1:80 (Fig. 1).

Toxoplasma gondii antibody titers in modified agglutination tests (MATs; Silva et al., 2002) of the 24 cats with NAT titers of 1:80 or more were generally low. The MAT titers of 18 cats with NAT titers of 1:80 were <1:25 in 9 cats, 1:25 in 4 cats, 1:50 in 4 cats, and 1:500 in 1 cat. The MAT titers of 5 cats with NAT titers of 1:160 were <25 in 2 cats, 1:25 in 2 cats, and 1:50 in 1 cat. The MAT titer of the cat

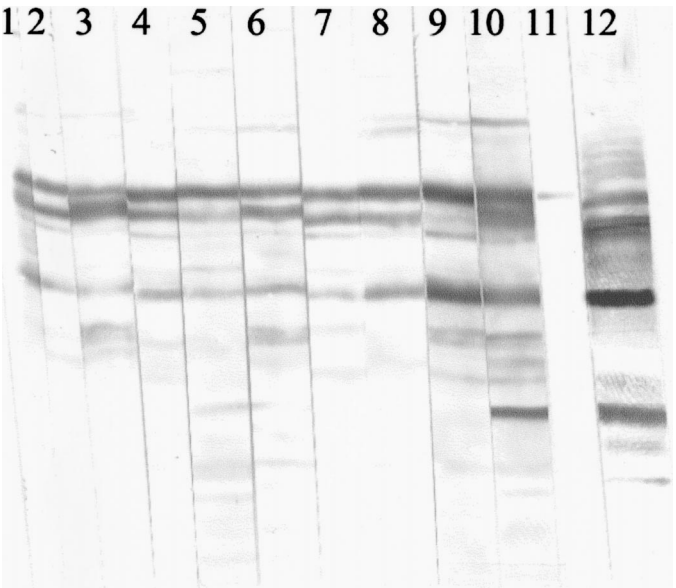

FIGURE 1. Western blot analysis of sera from 10 cats with antibody titers of 1:80 or higher in the NAT assay. Lanes 1–5, 1:80 NAT titer; Lanes 6–9, 1:160 titer; Lane 10, 1:800 titer; Lane 11, negative control cat sera; and Lane 12, positive control cat sera, 20 days postinfection with *Neospora caninum* tachyzoites.

with the highest NAT was 1:50. Thus, 10 of 24 cats with NAT titers of 1:160 or more had no demonstrable *T. gondii* antibodies, suggesting no cross reactivity between NAT and MAT (Romand et al., 1998).

Finding a high titer to *N. caninum* in at least 10 of the cats is of interest because there is no published case of *N. caninum* infection in domestic cats. During the course of the discovery of *N. caninum* in 1988, over 100 cats that died of *T. gondii*–like protozoan infection were examined, but *N. caninum* infection was not identified (Dubey and Carpenter, 1993; Dubey, unpubl.). However, experimentally infected cats can develop antibodies to *N. caninum* and clinical neosporosis (Dubey and Lindsay 1989a, 1989b; Dubey et al., 1990, 1996).

The NAT antibodies found in cats in the present study were not due to cross reactivity to *T. gondii* because antibodies to *T. gondii* were found in 26.3% of the same 502 cats tested in the *T. gondii* agglutination test (Silva et al., 2002).

Whether antibodies to *N. caninum* were due to past infection or cats had viable *N. caninum* infection is unknown because *N. caninum* has not yet been demonstrated in tissues of naturally infected cats. The NAT titer that should be considered specific for *N. caninum* infection in cats has not been determined. Therefore, all titers were given for cats. In cattle, even low titers (1:25) were considered indicative of exposure (Venturini et al., 1999), whereas Jenkins et al. (2000) considered the more conservative NAT titer of 1:80 as indicative of infection in cattle.

Cheadle et al. (1999) first reported *N. caninum* seropositivity in a small group of wild felids from Africa. They found low indirect fluorescent (1:50–1:200) antibody titers in 3 of 8 lions (*Panthera leo*) from Kruger National Park in South Africa and 1 of 16 cheetahs (*Acinonyx jubatus*) from Namibia.

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